

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/242567852>

Hepatitis B Vaccine: Immunity, Efficacy and Types

Article in Shiraz E Medical Journal · January 2010

CITATIONS

3

READS

946

2 authors:



Farahnaz Fallahian

Iran University of Medical Sciences

45 PUBLICATIONS 770 CITATIONS

[SEE PROFILE](#)



Farhad Zamani

Iran University of Medical Sciences

133 PUBLICATIONS 1,571 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Farahnaz Fallahian, Farhad Zamani, Seyed Mohammad Reza Hashemian. (2012) Hepatitis B Virus Genetic Diversity. 1: 209. Open Access Scientific Reports. doi:10.4172/scientificreports.209 [View project](#)



Chronic HBV [View project](#)

In the name of God

Shiraz E-Medical Journal
Vol. 11, No. 1, January 2010

<http://semj.sums.ac.ir/vol11/jan2010/88020.htm>

Hepatitis B Vaccine: Immunity, Efficacy and Types.

Fallahian F*, Zamani F*.

* Researcher, Gastroenterintestinal and Liver Disease Research Center, Iran University of Medical Sciences, Firuzgar Hospital, Tehran, Iran.

Correspondence: Dr. F. Fallahian, Gastroenterintestinal and Liver Disease Research Center, Iran University of Medical Sciences, Firuzgar Hospital, Vali asr Square, Aban St. Tehran, Iran. Telephone: +98(21) 8800-3264, Fax: +98(21) 8894-5188, E-mail: falahianfff@yahoo.com

Received for Publication: August 5, 2009, Accepted for Publication: September 29, 2009.

Abstract:

Current issues that are associated with the development of hepatitis B vaccine, combination vaccines, modes of administration, immunogenicity, and efficacy of different types of hepatitis B vaccines are reviewed. Hepatitis B viral mutants can emerge as a result of either immune response or treatment options. Several studies are in progress on treatment of chronic hepatitis B infection by immunization with multiple antigenic components; DNA vaccines alone or with DNA encoded immunomodulatory cytokines; combination of vaccine with antiviral drugs and cytokines; and genetic manipulation of antigen presenting cells. Integrating hepatitis B vaccine doses into the global infant immunization program is not sufficient for hepatitis B virus (HBV) infection eradication. Implementing HBV schedule to high risk groups such as injection drug users, inmates of correctional centers, and persons at risk for sexually transmitted diseases, surveillance of hepatitis B infected subjects and refugees, access to immunization services and treatment is necessary. Further investigation is needed to assess factors that can impede an adequate antibody response, HBV variants, and the need for booster doses to preserve vaccine-induced immunity, vaccinating schedule for older children, evaluation of those vaccinated but in persistent contact in HBV-endemic areas.

Keywords: Hepatitis B vaccine; Immunity; Efficacy; Booster policy; HBV mutants; Vaccination programs.

Introduction:

The prevalence of chronic hepatitis B virus infection varies by geographic region. Most of North America is a low-prevalence (< 2%) area. Certain high-prevalence pockets exist, especially areas with a high proportion of Asian immigrants and Alaskan and northern Canadian Aboriginal populations, where rates of chronic HBV are as high as 5% to 15%. In most low-prevalence areas, HBV infection is acquired mainly during adolescence and mid-adulthood, whereas perinatal transmission is the main route in high-prevalence (> or = 8%) areas.⁽¹⁾ Worldwide, mothers are the driving force behind the infections that lead to hepatocellular carcinoma (HCC), because the HBV carrier state is inversely proportional to the age of the infant when infected.⁽²⁾ Hepatitis B vaccines have been incorporated into national immunization programs in over 150 countries. The major humoral immune response is a common determinant of the surface antigen protein of the virus.⁽³⁾ Approximately 5-10% of healthy immunocompetent subjects do not mount an antibody response (anti-HBs). Non-response is associated with different HLA-DR alleles and impaired T helper (Th) cell response, among other factors such as route of injection, age, gender, and body mass. Studies are in progress on treatment of chronic hepatitis B infection by immunization with multiple antigenic components, combination of vaccine with antiviral drugs and cytokines, T cell vaccines, DNA vaccines alone or with DNA encoded immunomodulatory cytokines, and direct genetic manipulation of antigen presenting cells.⁽³⁾ Currently marketed HBV vaccines

make use of DNA recombinant technology by introducing the gene for HBsAg (S gene) into the genome of the yeast *Saccharomyces cerevisiae*. The two vaccines available are Recombinant HB and Engerix-B. The vaccines induce HBsAg-specific helper T cells and T cell-dependent B cells to produce neutralizing antibody against the "a" epitope (amino acid sequence 124-148) of HBsAg as early as 2 weeks after the first injection.^(4, 5)

A number of factors can impede an adequate antibody response. Smoking, obesity, injection into the buttock, chronic liver disease, presence of HLA-DR3, DR7, and DQ2 alleles, absence of the HLAA2 allele, and extremes of age may be associated with reduced immunogenicity. Response rates also are lower in immunocompromised patients, such as transplant recipients, patients receiving chemotherapy, and those with end-stage liver disease. Patients with chronic kidney disease should be vaccinated early in the course of their disease, before renal disease progresses, to ensure optimal response to vaccination. The HBV vaccine is administered to all infants and children as a part of the universal immunization program. Adolescents who have not been vaccinated in infancy or childhood should also be vaccinated. In immunocompromised patients and patients on dialysis, four vaccine doses are recommended.^(6, 7)

In this manuscript the history, efficacy, immunogenicity, and long-term protection, mutants and HBV variants, new vaccines, combination of vaccines, comparison of two HBV vaccines and two HBV

vaccines administration, new adjuvant, and vaccine safety will be reviewed.

Methods:

We performed a search conducted on hepatitis B vaccine immunity, efficacy, types, administration, and the impact of HBV vaccination on prevention of disease by using the electronic database MEDLINE (1982 to 2009), EMBASE, OVID, Google (for Local websites and medical journals), and Websites of Iranian universities.

History:

The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in randomly selected 6583 subjects from three provinces in Tehran, Golestan, and Hormozgan of Iran was 2.6% and 16.4%, respectively.⁽⁸⁾ The seroprevalence of confirmed HBsAg was 0.6% in specimens of 1,004,889 volunteer blood donors in Tehran blood transfusion service selected after interview and physical exam from 2003 to 2005⁽⁹⁾; 5% in sixty-six subjects with confirmed diagnosis of hemophilia⁽¹⁰⁾; 5.9% (CI 95%: 4.5–7.3%) in 1113 Iranian large vehicle drivers⁽¹¹⁾; 18% in 226 gypsies from Shahr-e-Kord southwest of Iran⁽¹²⁾; 10% in women and 14.2% in men who attended the laboratory for sexually transmitted diseases in the Northeast of Iran.⁽¹³⁾ In Iran, Cuban hepatitis B vaccine became available approximately in 1994 and mass vaccination of neonates and children was incorporated in the national vaccination scheme. In a study of 538 children who received three doses of Cuban hepatitis B vaccine: 15.6% were nonresponder (<10mIU/ml), 27.7% were

hyporesponder (10-100mIU/ml), and 56.7% were good responder (>100mIU/ml).⁽¹⁴⁾ Children who had been immunized with Cuban recombinant hepatitis B vaccine and their antibody titers were <10mIU/ml (nonresponder) or 10-100mIU/ml (hyporesponder), were administered booster dose of the same vaccine in the Deltoid muscle. The response of 141 children with the mean age of 1.9 years to booster dose of vaccine was 94.3% and 100% vaccines with the first and second booster dose of vaccination, respectively.⁽¹⁵⁾ Complete immunization coverage by age one year was better in rural areas than in urban areas. The reason for better coverage in rural areas was that village workers actively sought, visited and immunized children, while in urban areas physicians dominate care but do not often insist on immunization.⁽¹⁶⁾ Among 339 health care workers vaccinated with three doses of HBV vaccine in Shiraz, Iran, anti-HBsAb titers was 10 - 100 mIU/mL in 25.1%, and <10 mIU/mL of 12.7% of subjects. Reassessment for revaccination in health care workers should be considered.⁽¹⁷⁾ Mass campaign of immunization against hepatitis B registered for those born from 1989 to March 2007 in Iran. During this campaign 1,320,000 people were vaccinated and about 90% coverage was reached. In order to extend the target groups for hepatitis B vaccination, the program recommended also vaccinating people with high risk occupations like firefighters and workers at city hall. It was concluded that 12% of the first target group were already vaccinated against HBV.⁽¹⁸⁾

Universal infant hepatitis B immunization programs were initiated in Mongolia from 1991. A survey was conducted in 2004 on 593 children: the coverage of complete vaccination was 60.1%. A significantly higher proportion of children in Metropolitan cities (75.2%) were completely vaccinated compared to those in Province centers (55.7%) and rural areas (59.1%). Only 17.0% of the children had protective anti-HBs which decreased from 31.1% to 16.3% among 7 to 12-year-olds indicating its decay with time.⁽¹⁹⁾ Similarly, in Alaska and USA, the vaccine induced anti-HBs titer declined to 19% in the five years and 8% in the ten years of age.⁽²⁰⁾ The significant urban-rural differences in the prevalence of HBV infection and carriage suggest some potential gaps in the vaccination program implementation in remote areas. It should be noted that there was no significant differences in the risk of infection or in the frequency of HBV-infected mothers among urban and rural subjects.⁽²¹⁾ One explanation may be improper vaccine storage and handling led to vaccine damage during the cold winter season in Mongolia. It is well known that HBV vaccine loses its immunological potency upon freezing or freeze drying.⁽²²⁾ According to a model-based estimate, the annual number of HBV infections among 100,000 fully immunized children due to unsafe immunization injection was at least 135-3120. An insufficient supply of syringes, and the attitudes to justify reuse, were significantly associated with the unsafe reuse of a reusable syringe in most parts of the areas studied.⁽²³⁾

In Taiwan, a mass vaccination program for neonates born to HBsAg-positive

mothers was launched in 1984, followed by immunization of all neonates in 1986.⁽²⁴⁾ The average seroprevalence rate of hepatitis B surface antigen (HBsAg) in children aged less than 15 years fell from 9.8% in 1984 to 0.7% in 1999, with a similar-order drop in seroprevalence of anti-HBc from 20.6% to 2.9%.⁽²⁵⁾ The difference of long-term efficacy between plasma-derived and recombinant hepatitis B virus vaccines and the effectiveness of catch-up vaccination in adolescents with undetectable anti-HBs was investigated in Taiwan. Recombinant vaccine showed predominant disappearance rate (62.7%) of anti-HBs 12-15 years after vaccination, but provided better anamnestic response after a booster dose. It also showed high success rate (97.3%) in catch-up vaccination in adolescents.⁽²⁶⁾ The prevalence of occult HBV infection among forty-six HBsAg-negative HBV vaccinated children in Taiwan was 10.9% in at least two regions, with mean titer 1.60×10^4 copies/ml. Sequence analyses of S gene showed occult isolates were variants; no G145R but C139S vaccine escape mutant was found. Variation and deletion were found in pre-S region; pre-S deletion was more frequent in 3' terminus of pre-S1 which leads to loss of immune epitopes and function sites.⁽²⁷⁾

In 6213 subjects aged 6 months to 60 years from hospitals in Thailand, seropositive rates for HBsAg, anti-HBs, and anti-HBc was 4%, 41.6% and 26.5%, respectively. The HBsAg seropositive rate and anti-HBc was 0.7%, and 2.9% among children born after and 4.3%, and 15.8% among children born prior to HBV vaccine integration into the expanded

program on immunization.⁽²⁸⁾ Of eighty-seven high-risk individuals who had received a complete course of recombinant HBV vaccine 18-20 years ago in Thailand, cellular immunity was determined by ELISPOT to detect HBV-specific IFN-gamma-producing cells. Overall 58.6% were seroprotected (titer \geq 10 mIU/mL). As for cellular immunity, 50.6% were positive on ELISPOT. The majority of participants (81.8%) who were positive for IFN-gamma-producing cells were seropositive, but only 50% of seropositive participants were ELISPOT positive. Thus, 18-20 years after immunization, it appears that a second booster dose should be considered, especially in high-risk groups.⁽²⁹⁾ In a study in injecting drug users (IDUs) in Bangkok of Thailand, of the 189 HBV seronegative IDUs, 81.0% completed the vaccine series. IDUs with HIV had 6.5 time fold odds of vaccine non-response. These data underscore the need for, and feasibility of, vaccine delivery in this population and support targeting efforts at high-risk age groups.⁽³⁰⁾ Given the relatively high prevalence of HBV infection (9.7%) of 371 HIV-infected patients, screening for HBV infection prior to ART initiation should not be omitted in the resource-limited setting.⁽³¹⁾

Review of data of 1,485 HBsAg-positive pregnant in Alberta, Canada showed that of the 980 infants eligible to have completed prophylaxis and serological follow-up, 82.0% were appropriately immunized and serologically tested. Of infants with complete immunization and follow-up, 3.7% failed to mount an immune response and 2.1% were infected. Future research should examine maternal fac-

tors that may increase the vertical transmission of HBV.⁽³²⁾

In the United States from 1990 to 2001, the overall incidence of acute hepatitis B declined by 66%, from 8.1 to 2.8 cases per 100,000. The decline was most dramatic among children 0-11 years old, with an 89% decline, from 1.1 to 0.12 per 100,000.⁽³³⁾ Three-dose vaccination coverage for children aged 19-35 months increased from 16 percent in 1991 to 92 percent in 2004.⁽³⁴⁾ There has also been progress towards full implementation of routine immunization of adolescents not previously vaccinated. Vaccination coverage among adolescents aged 13-15 years increased from almost zero in 1993 to 74 percent in 2004. The introduction of laws requiring immunization prior to school entry has been observed to coincide with a rise in vaccination coverage.⁽³⁵⁾ Nearly all pregnant women in the United States receive prenatal HBsAg screening to identify those at risk of perinatal transmission to their infants.⁽³⁶⁾ On the basis of data from the 2004 National Health Interview Survey⁽³⁷⁾, hepatitis B vaccination coverage among all US adults (with and without indications for vaccination) is estimated to be 35 percent. The highest vaccination coverage rates among adults with an indication for vaccination have been observed among persons with potential occupational exposure to HBV. Vaccination coverage among staff members at dialysis centers in the United States was 90 percent in 2002, well above the 56 percent coverage reported among dialysis patients.⁽³⁸⁾ Data from the National Health Interview Survey showed that vaccination coverage among persons defined as "high-risk" for

sexually transmitted and blood borne diseases was only 30 percent in 2000 and 45 percent in 2004.⁽³⁹⁾ Findings of other studies are also consistent with low vaccination coverage among adults at risk for disease. Among 1,755 men who have sex with men (MSM) visiting a sexually transmitted disease clinic in San Diego, California in 1998, 16 percent were vaccinated. Of 1106 persons reporting injection drug use at the same clinic, only 6 percent were vaccinated.⁽⁴⁰⁾ A study conducted in San Diego in 1997 to examine adherence to vaccination recommendations among adult household and sexual contacts of persons with chronic hepatitis B, found that only 13 percent had received hepatitis B vaccine.⁽⁴¹⁾ The prevalence for HBV in the United States has been estimated to be approximately 0.4%. However, these estimates have been based on surveys conducted in samples in which foreign-born minorities were underrepresented. Voluntary screening data indicate prevalence in excess of 15% in some of these groups.⁽⁴²⁾ New HBV infections in the United States are becoming increasingly concentrated among populations such as injection drug users, inmates, and persons at risk for sexually transmitted diseases. Programs such as perinatal case management and venue-based vaccination of high-risk adults will need to be expanded and backed by a consistent commitment of resources.⁽⁴³⁾

Efficacy, immunogenicity and long-term protection

The persistence of anti-HBs depends on the peak antibody level achieved after three doses.⁽⁴⁴⁻⁴⁷⁾ Unresponsiveness to

HBsAg has been attributed to a number of environmental and genetic factors. The most important are the haplotype of HLA antigens and immunological tolerance.⁽⁴⁸⁾ A variety of HLA classes I and II antigens have been reported to be associated with unresponsiveness to HBsAg in different ethnic populations.⁽⁴⁹⁾ Strong B-cell responses in acute and chronic infection mediate neutralization of HBV and reduce the spread of virus, thus giving protective immunity. The weak T-helper cell and cytotoxic T lymphocyte responses in acute and chronic infection make for their limited role in immune control of viral replication and mean that core- and polymerase-specific T-cell immunity is crucial. Altogether, the conclusion is that HBV envelope antigens are suitable for use in protective vaccines or as adjuvants for therapeutic vaccine but not for use in therapeutic vaccines *per se*.^(50, 4) The third dose of vaccine in the infant immunization schedule produces a large and rapid rise in antibody such that it may be considered as itself a booster dose. By 10–15 years after the first immunization, anti-HBs titers have fallen to less than 10 mIU/ml in some 10–50% of those vaccinated. Clearly, protection against clinically important disease outlasts the presence of detectable antibodies. Revaccination after 10–13 years produced a response of >95%. The mechanistic basis of immune memory is evidently a complex interplay between memory B cells, memory T cells, memory cytotoxic T lymphocytes and antigen-antibody complexes.^(51- 52) In 2002, World Health Organization (WHO) repeated its view that booster doses of hepatitis B vaccine are not recommended for univer-

sal hepatitis B immunization program.⁽⁵³⁾ Immunocompromised subjects should be regularly tested for anti-HBs titres and given a hepatitis B booster vaccination when the titer fall below 10 mIU/ml.⁽⁵⁴⁾ Based on the currently available data there is no scientific evidence for giving booster doses of hepatitis B vaccine to fully immunized individuals. Although mutant hepatitis B viruses currently have little public health significance, regular monitoring is of importance to determine if vaccination fails to prevent infection by mutants. Should this occur, there might then be a need to revise immunization policies status.⁽⁵⁵⁾ Anti-HBs level in 19.3% of 3752 children who were vaccinated in three cities of Iran was <10 mIU/mL. The total geometric (GMT) mean titer was 34.5±0.66. Predictors were low birth weight and chronic disease.⁽⁵⁶⁾ A study evaluated the efficacy and immunogenicity of combined hepatitis B vaccination with plasma-derived vaccine (PDV) and recombinant vaccine (RV), and concluded four doses of vaccine are recommended in high-risk infants when two types of vaccine are to be used in combination.⁽⁵⁷⁾

In a recent study, in vitro production of interleukin (IL)-2, interferon (IFN)-gamma and IL-10 was investigated in Iranian healthy adults vaccinated with recombinant hepatitis B (rHB) vaccine. Peripheral blood mononuclear cells (PBMC) were isolated from 18 high responders and eight nonresponders and stimulated with rHB antigen or phytohaemagglutinin (PHA) mitogen. The results demonstrated a significant decrease in the production of IL-2, IFN-gamma and IL-10 in response to rHB antigen.

The findings suggest that unresponsiveness to rHB vaccine may be owing to inadequate Th1- and Th2-like cytokine production.⁽⁵⁸⁾

A study was conducted to evaluate the influence of supplementary vaccination with a single low and standard dose of a recombinant hepatitis B (HB) vaccine in healthy Iranian non-responder neonates to primary vaccination. The results indicate that a significant proportion of non-responder neonates can be induced to develop a protective anti-HBs response following revaccination with a single low dose vaccine.⁽⁵⁹⁾ A 3-year follow-up study of long-term antibody persistence following vaccination of low-risk preterm infants with recombinant hepatitis B vaccine (HBV), showed that delaying vaccination of premature infants against hepatitis B until a weight of 2,000 grams was reached, resulted in both a significantly higher percentage of children with positive antibody levels and a significantly higher geometric mean concentration (GMC) at 3-3.5 years of age as compared to early-vaccinated preterm infants.⁽⁶⁰⁾

Mutants, HBV variants

Hepatitis B viral mutants can emerge in patients as a result of selection pressure from either immune response or treatment options. Mutations that occur within the immunodominant epitopes of hepatitis B surface antigen (HBsAg) allow mutant virus to propagate in the presence of a neutralizing immune response, while wild-type virus is reduced to undetectable levels. An understanding of immunoassay reactivity with HBsAg mutants is key to establishing an appropriate testing

algorithm for hepatitis B virus detection programs.⁽⁶¹⁾

More than 20 years have elapsed since 1984, when vaccination against Hepatitis B began, first with a plasma-derived vaccine and later a recombinant DNA-derived vaccine. The appearance of Hepatitis B surface gene mutants in DNA HBV positive children has been confirmed, gradually increasing from 7.8% before vaccination to 23.1% 15 years later. To date, there is no evidence that those viruses are disrupting the efforts to control Hepatitis B through immunization programs.⁽⁶²⁾

Numerous mutant hepatitis B viruses have been identified, with various mutations having been detected in almost every part of the viral genome.⁽⁶³⁾ Some nucleotide changes allow the virus to escape the effects of vaccination and treatment. There have been reports of infants born to HBeAg positive mothers who developed breakthrough infections despite having undergone full passive-active immunoprophylaxis. Some of these instances have been attributed to strains of HBV with mutations in the antigenic determinant of HBsAg that result in weak binding to anti-HBs.⁽⁶⁴⁻⁶⁵⁾ A study in vaccinated children in Singapore⁽⁶⁶⁻⁶⁷⁾ showed that the antigen and antibody can coexist, and that mutants persisted for at least 13 years. Existing evidence suggests that HBsAg mutants may cause persistent infection and be associated with chronic hepatitis. They may also hinder detection of HBsAg in HBV infection. Mutants can infect unvaccinated subjects but whether they can infect vaccinated people is unclear. WHO

recommends the establishment of an independent global network for appropriate monitoring of such vaccine/ treatment-escape mutants.⁽⁶⁸⁻⁶⁹⁾ Vaccine failures due to HBV variants with mutations in the small surface protein (S) gene (S mutants) have occurred in perinatally exposed infants who received hepatitis B vaccine or hepatitis B immune globulin appropriately and who have concentrations of anti-HBs that are usually protective.⁽⁷⁰⁾ There has been concern that these HBV variants, which are sometimes resistant to the neutralizing effect of anti-HBs, could threaten the effectiveness of a hepatitis B immunization programs and that immunization may accelerate the formation of HBV variants.⁽⁷¹⁾

A study using sensitive detection methods demonstrated that the S mutants implicated in perinatally exposed infant vaccine failures were usually of maternal origin and not induced by vaccination.⁽⁷²⁾ In addition, mothers of infants who responded to vaccination were as likely to have these surface antigen variants as mothers of infants who did not respond, suggesting that infections among vaccinated children with S mutants represented immunoprophylaxis failures and infection with maternal viral variants rather than breakthrough infections among successfully vaccinated infants.⁽⁷³⁾ Most commercially available assays that employ polyclonal anti-HBs detect S mutants, making ongoing surveillance for S mutants possible.⁽⁷⁴⁾ Introduction of additional nucleoside/nucleotide analog agents for hepatitis B treatment will likely lead to the development of further unique polymerase mutants with varying

pathogenicity and cross-resistance to existing drugs.⁽⁷⁵⁾

New vaccines, combination of vaccines

Genetically engineered subunit vaccines are more costly to manufacture than conventional vaccines, since the antigen must be purified to a higher standard than was demanded of older, conventional vaccines. Recombinant subunit vaccines have a tendency to be less immunogenic than their conventional counterparts.⁽⁷⁶⁾ A study discussed the new field of application of antigen-pulsed dendritic cells (DCs) for prophylactic purposes when adequate levels of protective antibody cannot be induced by traditional vaccination approaches.⁽⁷⁷⁾

Combined hepatitis A and B vaccine, (Twinrix, GlaxoSmithKline Biologicals) is safe, well-tolerated and has demonstrated a highly immunogenic profile. Persistence of anti-HAV and anti-HBs antibodies in adults remains high for at least 10 years after primary vaccination.⁽⁷⁸⁾ All available data on monovalent and combined hepatitis A and hepatitis B vaccines indicates that there is no support for a hepatitis A or hepatitis B booster when a complete primary vaccination course is offered to immunocompetent individuals.⁽⁷⁹⁾

Plasma-derived vaccines and yeast-derived recombinant vaccines against HBV infection have gained an acceptable record of efficacy. However, non- or hyporesponsiveness to immunization does not only occur in cases of obesity, renal failure or immune suppression, but also in healthy individuals. There is a rationale

for developing more immunogenic vaccines against HBV, especially for those populations who are potential non- or hyporesponders. Currently used recombinant hepatitis B vaccines consist of antigen particles assembled with the product of 226 amino acids encoded in the S gene. Since proteins encoded in the pre-S gene are also incorporated in the HBV envelope, pre-S gene products should, at least in theory, be useful in improving protection with hepatitis B vaccines. Inactivated hepatitis A vaccines are more potent than currently used hepatitis B vaccines. Two injections of a standard dose of HAVRIX (SB) by the intramuscular route, or even a single injection using a higher dose (HAVRIX 1440), will achieve protective levels of antibodies. New hepatitis A vaccines are likely to be recombinant or attenuated live types.⁽⁸⁰⁾ A Study on 126 healthy 2-month-old infants that were given a bivalent Haemophilus influenzae type b/hepatitis B vaccine (bivalent Hib-HB vaccine; COMVAX) concurrently with priming doses of diphtheria-tetanus-pertussis vaccine (DTP), a booster dose of diphtheria-tetanus-acellular pertussis vaccine (DTaP), inactivated or oral polio vaccine (IPV or OPV) and measles-mumps-rubella vaccine (M-M-R(II)) confirmed that they have satisfactory antibody responses to all antigens. Concurrent administration of bivalent Hib-HB vaccine with priming doses of DTP, a booster dose of DTaP, OPV, IPV, or M-M-R (II) was well tolerated and, with the possible exception of rubella, did not substantially impair the antibody response to any antigen.⁽⁸¹⁾ In a study, four formulations of a liquid, hexavalent diphtheria-tetanus-acellular pertussis-

inactivated poliovirus-Haemophilus influenzae b conjugate-hepatitis B virus (DTaP-IPV-Hib-HBV) vaccine in 708 infants immunized at 2, 3, 4, and 12-14 months of age was compared. All vaccine formulations were well tolerated. The three PRP-OMPC formulations met pre-specified immunogenicity criteria, and the one with the lowest PRP-OMPC concentration was selected for further optimization of immunogenicity.⁽⁸²⁾

Compare of two HBV vaccines

A study compared two recombinant hepatitis B vaccines. One hundred seventy-three infants meeting eligibility criteria were given either GeneVac-B (Serum Institute of India Ltd.) or Engerix-B (GlaxoSmithKline Beecham) in a random fashion. The seroprotection rates were similar for both vaccines (96% and 95%, respectively). The study concluded that GeneVac-B is as immunogenic and as well tolerated as Engerix-B when administered with DTPw vaccine at 6, 10, and 14 weeks of age.⁽⁸³⁾

Compare of two HBV vaccines administration

To compare the efficacy of low-dose intradermal HBV recombinant vaccine with standard intramuscular dose in neonates, 165 apparently healthy neonates born in Shiraz, Iran were randomized to receive either recombinant (corrected dosage) vaccine intramuscularly or intradermally. Intradermal vaccination with 20% of standard dose is as effective as intramuscular vaccination when evaluated at 18 months after the first dose.⁽⁸⁴⁾ One to 10 per cent of healthy adult individuals do not produce protective levels of anti-

HBs antibodies, following a standard vaccination protocol. Lack of a HBs antigen (Ag)-specific T-cell repertoire is amongst the possible defects, which may lead to humoral unresponsiveness and is the main objective of this study. We analyzed TcR BV (T-cell receptor beta chain variable) gene usage in T lymphocytes from nine healthy adult responders and six nonresponders to recombinant HB vaccine, before and after booster vaccination. When the usage of each TcR BV gene within CD4+ and CD8+ T cells of the responders was compared with that of nonresponders, statistically significant difference was noted for BV5S2-3 gene family in CD4+ T cells of nonresponders. The results show that T-cell response to HBsAg is generally oligoclonal and involves multiple BV families. Furthermore, overexpressed individual TcR BV genes and CDR3 length distributions in response to HBsAg are subject-dependent.⁽⁸⁵⁾

A study concluded that the underlying causes of poor anti-HBs response in HBsAg carrier children who had cleared HBsAg but failed to develop anti-HBs after receiving three doses of HB vaccine, and subsequent one dose of the same vaccine, are multifactorial including specific failure of antigen presentation or T-cell activation, or the lack of T helper (Th)2 cell-like response to HBsAg. HLADrw14-DRw52 does not confer absolute nonresponsiveness to HBsAg.⁽⁸⁶⁾

In a randomized trial using a recombinant vaccine (Heberbiovac) to compare immunogenicity and safety of an intradermal low-dose (4 microg) with standard dose (20 microg) of intramuscular

vaccination in healthy Iranian population, the overall seroprotection rate (anti-HBs level \geq 10 IU/L) was 97.3% for intradermal vaccination group, which was not different from that of intramuscular vaccination group (98.55%). GMT of anti-HBs was not significantly different between the two groups.⁽⁸⁷⁾

New adjuvant

There have been promising developments in the area of new adjuvant and delivery systems. The practical need for reducing the total number of childhood vaccinations has driven development of, multivalent and combination vaccine formulations. Efforts have been made in the critical area of therapeutic application of the vaccine.⁽⁸⁸⁾

A study evaluated the ligands for Toll-like receptor 7 (TLR7) (R-848) and TLR 9 (CpG ODN) as adjuvants to augment the cellular and humoral immune responses as well as the generation of long-lasting immune memories following the vaccination with HBsAg in mice. The immune responses were assessed by enzyme-linked immunosorbent assay (ELISA), enzyme-linked immunospot (ELISPOT), and fluorescence-activated cell sorter (FACS) at the total and single-cell levels. The results imply that CpG ODN and R-848 may be the candidates as adjuvants for use in prophylactic and therapeutic hepatitis B vaccine.⁽⁸⁹⁾ The efficacy of granulocyte macrophage colony-stimulating factor (GM-CSF) to enhance the immune response to hepatitis B virus vaccine has been the subject of several reports. According to a study, GM-CSF induced a significant effect in terms of

response rate and achievement of an earlier seroconversion to the vaccine in the overall populations examined (renal failure patients and in healthy individuals).⁽⁹⁰⁾

CPG 7909 is an oligodeoxynucleotide containing immunostimulatory CpG motifs that activate human B and plasmacytoid dendritic cells via Toll-like receptor 9. The immunostimulatory properties of CPG 7909 present an important strategy in achieving longterm protection in HIV-infected patients and other HBV vaccine-hyporesponsive populations.⁽⁹¹⁾

Vaccine safety

At present, there are no data to conclude that hepatitis B vaccines pose a serious health risk or justify a change in current immunization practice. However, vaccine "scare" continues to have an international impact on immunization coverage. Creating a positive environment for immunization can be achieved by repositioning the value of vaccines and vaccination, supported by evidence-based information. The role of international organizations, the media, and the industry in the implementation of communication strategies was discussed and the impact of litigation issues on vaccinations were evaluated. The Viral Hepatitis Prevention Board confirms its commitment to current recommendations for universal and risk group hepatitis B vaccination and further encourages the conduct of vaccine safety studies and the dissemination of their results.⁽⁹²⁾ Lichen planus is a pruritic inflammatory dermatosis of unknown origin. An increased prevalence of a wide range of liver disease in lichen planus has

been observed by many authors. Most recently, many reports observed the occurrence of lichen planus after administration of different types of hepatitis B vaccines.⁽⁹³⁾

In conclusion:

Intensifying HB vaccination of high risk groups, surveillance of hepatitis B infected subjects, and control on the health status of refugees will further decrease the frequency of the disease. In addition the consideration of all possible routes of transmission in subjects without risk factors for infection is necessary.⁽⁹⁴⁾ Advisory Committee on Immunization Practices (ACIP) recommends universal hepatitis B vaccination for all unvaccinated adults. In other primary care and specialty medical settings in which adults at risk for HBV infection receive care, health-care providers should inform all patients about the health benefits of vaccination, including risks for HBV infection and persons for whom vaccination is recommended, and vaccinate adults who report risks for HBV infection and any adults requesting protection from HBV infection.⁽⁹⁵⁾ Causes of vaccine failure and HBV variants need to be assessed. To eliminate HBV transmission, global infant immunization programs and specific populations at high risk for HBV exposure have to be made. Access to settings for health seeking behavior and preventive care and immunization services and treatment are necessary for populations mentioned below:

-Persons at risk for sexual transmission, sex partners of persons who are positive for hepatitis B surface antigen (HBsAg),

persons evaluated or treated for sexually transmitted diseases, including human immunodeficiency virus infection, men who have sex with men

- Persons at risk for transmission by percutaneous or mucosal exposure to blood

- Household contacts of HBsAg-positive persons

-Current or recent injection drug users, including needle-sharing contacts of HBsAg-positive persons

-Health care and public safety workers with a reasonably anticipated risk of exposure to blood or blood-contaminated body fluids

-Persons with end-stage renal disease, including predialysis, hemodialysis, peritoneal dialysis, and home dialysis patients

- International travelers to areas with high or intermediate levels of endemic hepatitis B virus infection (HBsAg prevalence 2%)

-Persons with chronic liver disease

-Correctional facilities

-All persons seeking protection from hepatitis B virus infection

HLA= Human Leukocyte antigens

mIU/mL = milli international units per milliliter

References:

1. Carey WD. The prevalence and natural history of hepatitis B in the 21st century. *Cleve Clin J Med* 2009;76 Suppl 3: S2-5.
2. Beasley RP. Rocks along the road to the control of HBV and HCC. *Ann Epidemiol* 2009; 19 (4): 231-4.
3. Zuckerman JN. Protective efficacy, immunotherapeutic potential, and safety of hepatitis B vaccines. *J Med Virol* 2006; 78 (2): 169-77.
4. Böcher WO, Herzog-Hauff S, Herr W, Heermann K, Gerken G, Meyer Zum Büschenfelde KH, Löhr HF. Regulation of the neutralizing anti-hepatitis B surface (HBs) antibody response in vitro in HBs vaccine recipients and patients with acute or chronic hepatitis B virus (HBV) infection. *Clin Exp Immunol* 1996; 105 (1): 52-8.
5. Böcher WO, Herzog-Hauff S, Schlaak J, Meyer zum Büschenfeld KH, Löhr HF. Kinetics of hepatitis B surface antigen-specific immune responses in acute and chronic hepatitis B or after HBs vaccination: stimulation of the in vitro antibody response by interferon gamma. *Hepatology* 1999; 29 (1): 238-44.
6. Seaworth B, Drucker J, Starling J, Drucker R, Stevens C, Hamilton J. Hepatitis B vaccines in patients with chronic renal failure before dialysis. *J Infect Dis* 1988; 157 (2): 332-7.
7. Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991; 40 (RR-13): 1-25.
8. Merat S, Rezvan H, Nouraie M, Jamali A, Assari S, Abolghasemi H, et al. The prevalence of hepatitis B surface antigen and anti-hepatitis B core antibody in Iran: a population-based study. *Arch Iran Med* 2009; 12 (3): 225-31.
9. Khedmat H, Alavian SM, Miri SM, Amini M, Abolghasemi H, Hajibeigi B, et al. Trends in Seroprevalence of Hepatitis B, Hepatitis C, HIV, and Syphilis Infections in Iranian Blood Donors from 2003 to 2005. *Hepatitis Monthly* 2009; 9: 24-28.
10. Mohammad Alizadeh AH, Rezazadeh M, Ranjbar M, Donboli K, Fallahian F, Hadjilooi M, et al. Frequency of hepatitis B and hepatitis C infections and its association with development of factor VIII inhibitor in hemophiliacs in Hamadan Province of Iran. *International Journal of Medicine and Medical Sciences* 2009; 1 (5): 173-177.
11. Jahani MR, Motevalian SA, Mahmoodi M. Hepatitis B carriers in large vehicle drivers of Iran. *Vaccine* 2003; 21: 1948-1951.
12. Hosseini Asl SK, Avijgan M, Mohamadnejad M. High prevalence of HBV, HCV, and HIV infections in Gypsy population residing in Shahr-E-Kord. *Arch Iran Med* 2004; 7: 20 - 22.
13. Ghanaat J, Sadeghian A, Ghazvini K, Nassiri MR. Prevalence and risk factors for hepatitis B virus infections among STD patients in northeast region of Iran. *Med Sci Monit* 2003; 9: CR91-94.
14. Dahifar. H. Immunogenicity of Cuban Hepatitis B vaccine in Iranian children. *Arch Iranian Med* 2004; 7 (2): 89 - 92.
15. Dahifar. H, Mousavi. F, Ghorbani. A: Response of Booster Dose of Cuban Recombinant Hepatitis-B Vaccine in Nonresponder and Hyporesponder Children. *Pak J Med Sci* 2007; 23 (1): 23-26.
16. Nasser K, Sadrizadeh B, Malek-Afzali H, Mohammad K, Chamsa M, Cheraghchi-Bashi MT, et al. Primary health care and immunisation in Iran. *Public Health* 1991; 105: 229-238.
17. Saberifiroozi M, Gholamzadeh S, Serati AR. The long-term immunity among health care workers vaccinated against hepatitis B virus in a large referral hospital in southern Iran. *Arch Iran Med* 2006; 9 (3): 204-7.
18. Alavian. SM. Ministry of health in Iran is serious about controlling hepatitis B. *Hepatitis Monthly* 2007; 7 (1): 3-5.
19. Davaalkham D, Ojima T, Uehara R, Watanabe M, Oki I, Wiersma S, Nymadawa P, Nakamura Y. Impact of the universal hepatitis B immunization program in Mongolia: achievements and challenges. *J Epidemiol* 2007; 17 (3): 69-75.
20. Dentinger CM, McMahon BJ, Butler JC, Dunaway CE, Zanis CL, Bulkow LR, et al. Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. *Pediatr Infect Dis J* 2005; 24: 786-92.
21. Davaalkham D, Ojima T, Wiersma S, Lkhagvasuren Ts, Nymadawa P, Uehara R, Watanabe M, Oki I, Takahashi M, Okamoto H, Nakamura Y. Administration of hepatitis B vaccine in winter as a significant predictor of poor vaccination effectiveness in rural Mongolia: Evidence from nationwide survey. *J Epidemiol Community Health* 2007; 61 (7): 578-84.

22. Wilson N, Ruff TA, Rana BJ, Leydon J, Locarnini S. The effectiveness of the infant hepatitis B immunization program in Fiji, Kiribati, Tonga and Vanuatu. *Vaccine* 2000; 18: 3059-66.
23. Murakami H, Kobayashi M, Zhu X, Li Y, Wakai S, Chiba Y. Risk of transmission of hepatitis B virus through childhood immunization in northwestern China. *Soc Sci Med* 2003; 57 (10): 1821-32.
24. Safary A, Beck J. Vaccination against hepatitis B: current challenges for Asian countries and future directions. *J Gastroenterol Hepatol* 2000; 15 (4): 396-401.
25. Ni YH, Chang MH, Huang LM, Chen HL, Hsu HY, Chiu TY, et al. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med* 2001; 135 (9): 796-800.
26. Kao JT, Wang JH, Hung CH, Yen YH, Hung SF, Hu TH, et al. Long-term efficacy of plasmaderived and recombinant hepatitis B vaccines in a rural township of Central Taiwan. *Vaccine*. 2009 Mar 13; 27 (12): 1858-62.
27. Mu SC, Lin YM, Jow GM, Chen BF. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. *J Hepatol* 2009; 50 (2): 264-72.
28. Chongsrisawat V, Yoocharoen P, Theamboonlers A, Tharmaphornpilas P, Warinsathien P, Sinlaparatsamee S, et al. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health* 2006; 11 (10):1496-502.
29. Chinchai T, Chirathaworn C, Praianantathavorn K, Theamboonlers A, Hutagalung Y, Bock PH, et al. Long-term humoral and cellular immune response to hepatitis B vaccine in high-risk children 18-20 years after neonatal immunization. *Viral Immunol* 2009; 22 (2): 125-30.
30. Sunthornchart S, Linkins RW, Natephisarnwanish V, Levine WC, Maneesinthu K, Lolekha R, et al. Prevalence of hepatitis B, tetanus, hepatitis A, human immunodeficiency virus and feasibility of vaccine delivery among injecting drug users in Bangkok, Thailand, 2003-2005. *Addiction* 2008; 103 (10): 1687-95.
31. Sungkanuparph S, Wongprasit P, Manosuthi W, Atamasirikul K. Compliance with hepatitis B and hepatitis C virus infection screening among HIV-1 infected patients in a resource-limited setting. *Southeast Asian J Trop Med Public Health* 2008; 39 (5): 863-6.
32. Plitt SS, Somily AM, Singh AE. Outcomes from a Canadian public health prenatal screening program for hepatitis B: 1997-2004. *Can J Public Health* 2007; 98 (3): 194-7.
33. Centers for Disease Control and Prevention. Hepatitis Surveillance Report No. 59. Atlanta, GA, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, 2004. http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/hep_surveillance_59.pdf (Accessed January 2005).
34. Yusuf H, Daniels D, Mast EE, Coronado V. Hepatitis B vaccination coverage among United States children. *Pediatr Infect Dis J* 2001; 20 (11 Suppl): S30-3.
35. Effectiveness of a middle school vaccination law—California, 1999–2001. *MMWR Morb Mortal Wkly Rep* (2001) 50: 660–3.
36. Schrag SJ, Arnold KE, Mohle-Boetani JC, Lynfield R, Zell ER, Stefonek K, et al. Prenatal screening for infectious diseases and opportunities for prevention. *Obstet Gynecol* 2003 102 (4): 753-60.
37. Hepatitis B vaccine coverage among adults—United States, 2004. *MMWR Morb Mortal Wkly Rep* (2006) 55: 509–11.
38. Finelli L, Miller JT, Tokars JJ, Alter MJ, Arduino MJ. National surveillance of dialysis-associated diseases in the United States. *Semin Dial* 2005; 18 (1): 52-61.
39. Jain N, Yusuf H, Wortley PM, Euler GL, Walton S, Stokley S. Factors associated with receiving hepatitis B vaccination among high-risk adults in the United States: an analysis of the National Health Interview Survey, 2000. *Fam Med* 2004; 36 (7): 480-6.
40. Hepatitis B vaccination among high-risk adolescents and adults—San Diego, California, 1998–2001. *MMWR Morb Mortal Wkly Rep* (2002) 51: 618–21.
41. Weinberg MS, Gunn RA, Mast EE, Gresham L, Ginsberg M. Preventing transmission of hepatitis B virus from people with chronic infection. *Am J Prev Med*. 2001 May; 20 (4): 272-6.
42. Kim WR. Epidemiology of hepatitis B in the United States. *Hepatology* 2009;49 (5 Suppl): S28-34.

43. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B Virus Infection: Epidemiology and Vaccination. *Epidemiologic Reviews* 2006; 28 (1): 112-125. *Epidemiol Rev* 2006; 28: 112-25.
44. Hadler SC, Francis DP, Maynard JE, Thompson SE, Judson FN, Echenberg DF, et al. Longterm immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *N Engl J Med* 1986; 315: 209 – 14.
45. Tilzey A, Palmers SI, Banatvala JE, Vines SK, Gilks WR. Hepatitis B vaccine boosting among young healthy adults. *Lancet* 1994; 344: 1438 – 9.
46. Amani A, Shokri F. Immunogenicity of recombinant hepatitis B vaccine in Iranian neonates. *Iranian J Med Sci* 1995; 20: 87–92.
47. Hadler SC, Margolis HS. Hepatitis B immunization: vaccine types, efficacy, and indications for immunization. *Curr Clin Top Infect Dis.* 1992; 12: 282-308.
48. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Ann Rev Immunol* 1995; 13: 29 –60.
49. Martinetti M, Cuccia M, Daielli C, Ambroselli F, Gatti C, Pizzochero C, et al. Anti-HBV neonatal immunization with recombinant vaccine. Part II. Molecular basis of the impaired alloreactivity. *Vaccine* 1995; 13: 555 – 60.
50. Maruyama T, McLachlan A, Iino S, Koike K, Kurokawa K, Milich DR. The serology of chronic hepatitis B infection revisited, *J Clin Invest* 1993; 91 (6): 2586–2595.
51. Mele A, Tancredi F, Romanò L, Giuseppone A, Colucci M, Sangiuolo A, et al. Effectiveness of hepatitis B vaccination in babies born to hepatitis B surface antigen-positive mothers in Italy. *J Infect Dis* 2001; 184 (7): 905-8.
52. Wainwright RB, Bulkow LR, Parkinson AJ, Zanis C, McMahon BJ. Protection provided by hepatitis B vaccine in a Yupik Eskimo population – results of a 10-year study. *J Infect Dis* 1997; 175 (3): 674-7.
53. World Health Organization. Core information for the development of immunization policy. Document WHO/V&B/02.28, 2002. Geneva, WHO, 2002.
54. European Consensus Group on Hepatitis B Immunity. Are booster immunisations needed for lifelong hepatitis B immunity? *Lancet* 2000; 355: 561–5.
55. Fitzsimons D, François G, Hall A, McMahon B, Meheus A, Zanetti A, Duval B, Jilg W, Böcher WO, Lu SN, Akarca U, Lavanchy D, Goldstein S, Banatvala J, Damme PV. Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus mutants. *Vaccine* 2005; 23 (32): 4158-66.
56. Hassan S, Ziba F. Antibody titer in Iranian children 6 years after hepatitis B vaccine administration. *Vaccine* 2007; 25(17): 3511-4.
57. Lee PI, Lee CY, Huang LM, Chen JM, Chang MH. A follow-up study of combined vaccination with plasma-derived and recombinant hepatitis B vaccines in infants. *Vaccine* 1995; 13 (17): 1685-9.
58. Kardar GA, Jeddi-Tehrani M, Shokri F. Diminished Th1 and Th2 cytokine production in healthy adult nonresponders to recombinant hepatitis B vaccine. *Scand J Immunol.* 2002; 55 (3): 311-4.
59. Jafarzadeh A, Zarei S, Shokri F. Low dose revaccination induces robust protective anti-HBs antibody response in the majority of healthy non-responder neonates. *Vaccine* 2008; 26 (2): 269-76.
60. Linder N, Vishne TH, Levin E, Handsheer R, Fink-Kremer I, Waldman D, Levine A, Ashkenazi S, Sirota L. Hepatitis B vaccination: long-term follow-up of the immune response of preterm infants and comparison of two vaccination protocols. *Infection* 2002; 30 (3): 136-9.
61. Coleman PF. Detecting hepatitis B surface antigen mutants. *Emerg Infect Dis.* 2006; 12 (2): 198-203.
62. Vildozola H. Vaccination against Hepatitis B: 20 years later. *Rev Gastroenterol Peru* 2007; 27 (1): 57-66.
63. François G, Kew M, Van Damme P, Mphahlele MJ, Meheus A. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? *Vaccine* 2001; 19 (28-29): 3799-815.
64. Cooreman MP, van Roosmalen MH, te Morsche R, Sünner CM, de Ven EM, Jansen JB, Tytgat GN, de Wit PL, Paulij WP. Characterization of the reactivity pattern of murine monoclonal antibodies against wild-type hepatitis B surface antigen to G145R and other naturally occurring "a" loop escape mutations. *Hepatology* 1999; 30(5): 1287-92.
65. Shizuma T, Hasegawa K, Ishikawa K, Naritomi T, Iizuka A, Kanai N, et al. Molecular analysis of antigenicity and immunogenicity of a vaccine-induced escape mutant

of hepatitis B virus. *J Gastroenterol* 2003; 38 (3): 244-53.

66. Carman WF, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, et al. Vaccine-induced escape mutant of hepatitis B virus. *Lancet*. 1990 Aug 11; 336 (8711): 325-9.

67. Oon CJ, Chen WN. Current aspects of hepatitis B surface antigen mutants in Singapore. *J Viral Hepat* 1998; 5 Suppl 2: 17-23.

68. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000; 355 (9213): 1382-4.

69. World Health Organization. Hepatitis B – HBV mutants. WHO Department of Communicable Disease Surveillance and Response, CSR, 2004. http://www.who.int/emcdocuments/hepatitis/docs/whocdscsrlyo20022/virus/hbv_mutants.html (Accessed January 2005.)

70. Hsu HY, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; 30 (5): 1312-7.

71. Carman WF. The clinical significance of surface antigen variants of hepatitis B virus. *J Viral Hepat*. 1997; 4 Suppl 1: 11-20.

72. Ngui SL, O'Connell S, Eglin RP, Heptonstall J, Teo CG. Low detection rate and maternal provenance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxis in England and Wales. *J Infect Dis* 176 (5): 1360-5.

73. Nainan OV, Khristova ML, Byun K, Xia G, Taylor PE, Stevens CE, et al. Genetic variation of hepatitis B surface antigen coding region among infants with chronic hepatitis B virus infection. *J Med Virol* 2002; 68: 319-27.

74. Hussain M, Chu CJ, Sablon E, Lok AS. Rapid and sensitive detection assays for determination of hepatitis B virus (HBV) genotypes and detection of HBV precore and core promoter variants. *J Clin Microbiol* 2003; 41: 3699-705.

75. Wai CT, Fontana RJ. Clinical significance of hepatitis B virus genotypes, variants, and mutants. *Clin Liver Dis*. 2004; 8(2): 321-52.

76. Dertzbaugh MT. Genetically engineered vaccines: an overview. *Plasmid* 1998; 39(2):100-13.

77. Fazle Akbar SM, Furukawa S, Yoshida O, Hiasa Y, Horiike N, Onji M. Induction of anti-HBs in HB vaccine nonresponders in vivo by hepatitis B surface antigen-pulsed blood dendritic cells. *J Hepatol* 2007; 47 (1): 60-6.

78. Van Herck K, Leroux-Roels G, Van Damme P, Srinivasa K, Hoet B. Ten-year antibody persistence induced by hepatitis A and B vaccine (Twinrix) in adults. *Travel Med Infect Dis* 2007; 5 (3): 171-5.

79. Van Damme P, Van Herck K. A review of the long-term protection after hepatitis A and B vaccination. *Travel Med Infect Dis* 2007; 5 (2): 79-84.

80. Iwarson S. New approaches to hepatitis A and B vaccines. *APMIS*. 1995 May; 103 (5): 321-6.

81. West DJ, Rabalais GP, Watson B, Keyserling HL, Matthews H, Hesley TM. Antibody responses of healthy infants to concurrent administration of a bivalent haemophilus influenzae type b-hepatitis B vaccine with diphtheria-tetanus-pertussis, polio and measles-mumps-rubella vaccines. *BioDrugs* 2001; 15 (6): 413-8.

82. Halperin SA, Tapiero B, Diaz-Mitoma F, Law BJ, Hoffenbach A, Zappacosta PS, Radley D, McCarson BJ, Martin JC, Brackett LE, Boslego JW, Hesley TM, Bhuyan PK, Silber JL. Safety and immunogenicity of a hexavalent diphtheria-tetanus-acellular pertussis-inactivated poliovirus-Haemophilus influenzae b conjugate-hepatitis B vaccine at 2, 3, 4, and 12-14 months of age. *Vaccine* 2009; 27 (19): 2540-7.

83. Shivananda , Somani V, Srikanth BS, Mohan M, Kulkarni PS. Comparison of two hepatitis B vaccines (GeneVac-B and Engerix-B) in healthy infants in India. *Clin Vaccine Immunol* 2006; 13 (6): 661-4.

84. Lankarani KB, Taghavi A R, Agah S , Karimi A: Comparison of intradermal and intramuscular administration of hepatitis B vaccine in neonates. : *Indian J Gastroenterol* 2001; 20 (3): 94-6.

85. Soroosh. P, Shokri. F, Aziziz YM, M. Jeddi-Tehrani. M: Analysis of T-cell receptor Beta chain variable gene segment usage in healthy adult responders and nonresponders to recombinant hepatitis B vaccine. *Scand J Immunol* 2003; 57 (5): 423-31.

86. Hsu HY, Chang MH, Hsieh RP, Ni YH, Chi WK. Humoral and cellular immune responses to hepatitis B vaccination in hepatitis B surface antigen-carrier children who cleared serum-hepatitis B surface antigen. *Hepatology* 1996; 24 (6): 1355-60.

87. Ghabouli MJ, Sabouri AH, Shoeibi N, Bajestan SN, Baradaran H. High seroprotection rate induced by intradermal administration of a recombinant hepatitis B vaccine in young healthy adults: comparison with standard intramuscular vaccination. *Eur J Epidemiol* 2004; 19 (9): 871-5.
88. Sanyal G, Shi L. A review of multiple approaches towards an improved hepatitis B vaccine. *Expert Opin Ther Pat* 2009; 19 (1): 59-72.
89. Ma R, Du JL, Huang J, Wu CY. Additive effects of CpG ODN and R-848 as adjuvants on augmenting immune responses to HBsAg vaccination. *Biochem Biophys Res Commun* 2007; 361 (2): 537-42.
90. Cruciani M, Mengoli C, Serpelloni G, Mazzi R, Bosco O, Malena M. Granulocyte macrophage colony-stimulating factor as an adjuvant for hepatitis B vaccination: a meta-analysis. *Vaccine* 2007; 25 (4): 709-18.
91. Cooper CL, Angel JB, Seguin I, Davis HL, Cameron DW. CPG 7909 adjuvant plus hepatitis B virus vaccination in HIV-infected adults achieves long-term seroprotection for up to 5 years. *Clin Infect Dis* 2008; 46 (8): 1310-4.
92. François G, Duclos P, Margolis H, Lavanchy D, Siegrist CA, Meheus A, Lambert PH, Emiroğlu N, Badur S, Van Damme P. Vaccine safety controversies and the future of vaccination programs. *Pediatr Infect Dis J*. 2005; 24 (11): 953-61.
93. Al-Khenaizan S. Lichen planus occurring after hepatitis B vaccination: a new case. *J Am Acad Dermatol*. 2001; 45(4): 614-5.
94. Alavian SM, Fallahian F, Lankarani KB: The changing epidemiology of viral hepatitis B in Iran. *J Gastrointest Liver Dis*. December 2007; 16 (4): 403-406.
95. Mast EE, Weinbaum CM, Fiore AE, Alter MJ, Bell BP, Finelli L, et al. Advisory Committee on Immunization Practices (ACIP) Centers for Disease Control and Prevention (CDC). A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part II: immunization of adults. *MMWR Recomm Rep*. 2006 Dec 8; 55 (RR-16): 1-33; quiz CE1-4.